

DISTANCE DECAY ANALYSIS OF THE ALKALIPHILIC BACTERIA ISOLATED FROM LONAR SODA LAKE

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Abstract

In the present study distance wise microbial diversity (Distance Decay Analysis) of five different soil samples from Lonar lake region was studied. The first sample of soil was collected from the bank of Lonar Lake and remaining four sediment samples were collected from 2, 4, 6, and 8 meter away from the bank respectively. Total eight Alkaliphilic isolates were isolated from these samples using Horikoshi agar (pH 11-7). The isolates were labeled as BC1-*Pseudomonas* species, BC2-*Staphylococcus* species, and BC3-*Streptomyces* species and five species (BC4-BC8) of *Bacillus* species. All the isolates followed the Distance Decay Principle. The count (CFU/mL) of isolates BC1, BC2, BC3, BC5 and BC6 was increased as the distance increased from the bank of the lake that is from sample-I to sample-V. This denotes that the species BC1-*Pseudomonas* species, BC2-*Staphylococcus* species, BC3-*Streptomyces* species, BC5-*Bacillus subtilis*, and BC6-*Bacillus licheniformis* were less adapted to alkaline environment moving away from the Lake. On the other hand isolates BC4-*Bacillus circulans*, BC7-*Bacillus megaterium* and BC8-*Bacillus alvei* showed decrease in bacterial count (CFU/mL) from soil sample I-V. These three species showed resistance against the alkaline conditions and adapted near to the bank of the Lake while all isolates were tolerant to 8% NaCl.

Key words: Lonar Lake; Distance Decay; Biodiversity; Alkaliphililes.

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INTRODUCTION

The first law of geography states that, the similarity between two observations often decreases or decays as the distance between them increases (Tobler, 1970). Distance decay is a geographical term which describes the effect of distance on cultural or spatial interactions. The distance decay effect states that the interaction between two locales declines as the distance between them increases. Related terms include "friction of distance," which describes the force that creates distance decay, also called as first law of geography, an informal statement that "All things are related, but near things are more related than far things."

Lonar is one of the famous soda lakes situated in the Buldhana district of Maharashtra in India. It is almost circular, with its longest and shortest diameter being 1875m and 1787m respectively with a rim of about 30m and a depth of 135m. It is the third largest crater in the world and the only known crater formed by meteoritic impact in basaltic rock (Touche *et al.*, 1912). This lake contains high concentrations of sodium carbonate and complexes of this salts formed by evaporation of water. Due to this high concentration of sodium carbonate and its complexes the pH of the water reached to 11.5. Soda Lake represents the most stable naturally occurring alkaline environment on earth. These soda lakes contain water of very high pH due to presence of salts like sodium carbonates. Therefore these lakes represent a unique ecological niche for many organisms (Wani *et al.*, 2006). Microorganisms are omnipotent and omnipresent and soda lakes have been extensively studied for their microbial diversity (Tambekar *et al.*, 2012). Generally this lake contains microorganisms which grow at high pH called as Alkaliphiles.

The perspective of the present study focuses on the adaptation of the Alkaliphiles with changing environmental conditions of the Lonar Lake. It gives the estimate of the migration pattern of the Alkaliphilic bacteria towards the more favorable conditions. The regression of similarity against distance unites several ecological phenomena, and thus provides a highly useful approach for illustrating the spatial turnover across sites. The present study attempted to analyze whether Distance Decay (First Law of Geography) was applicable or not for Alkaliphilic bacteria from Lonar Soda Lake and it hypothesized that the Alkaliphilic bacteria with lower dispersal ability would show higher distance decay rates than a group with higher dispersal ability. The useful information about the ecology of the Alkaliphilic of the Lonar Lake was explored in the study.

MATERIALS AND METHODS

Collection of samples

A total of 5 locations were selected for five different soil samples from Lonar Lake situated at Lonar, District Buldhana, (M.S) India. The first sample of soil was collected from the bank of Lonar Lake and remaining four sediment samples were collected from 2, 4, 6, and 8 m away from the bank respectively. Soil samples were collected in sterile screw cap test tubes from 5cm depth from each location using forceps. pH and temperature was noted at the time of sampling. Soil samples were then carried out to the Department of Microbiology, New Arts, Commerce and Science College, Ahmednagar.

Isolation and Identification of bacterial culture

The sediment samples were subjected to isolation using conventional spread plate technique on Horikoshi medium containing (g/L) Glucose-10, Peptone- 5, Yeast extract -5, KH_2PO_4 -1, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -0.2, Na_2CO_3 -10, Agar- 20. The pH of the medium was adjusted to 11.7. Isolated bacteria were sub-cultured on Horikoshi Agar slants for further use (Horikoshi K, 1971). All the isolates were identified on the basis of standard cultural, morphological, biochemical characteristics and sugar fermentation tests according to Bergey's Manual of Determinative Bacteriology (9th Edition).

Analysis of Distance Decay using Simpsons Index of Diversity

Simpson's Index of Diversity (1-D) to analyze the Distance Decay Hypothesis was calculated for each isolate using the formula as,

Simpson's Index of Diversity =1-D

Where,

D is Simpsons diversity Index and is calculated as

$$D = \sum P_i^2$$

Where $P_i = B/Q$

B = mean number of colonies of one type

Q= mean of total number of colonies.

RESULTS AND DISCUSSION

While studying the distance decay analysis for the Alkaliphilic bacteria from alkaline Lonar Lake, a total of five soil samples were collected. The first sample collected from immediate bank of the Lake showed pH-11 whereas the pH for rest four samples showed decreasing trend as the sampling proceeded away from the bank (pH 11.7). The temperature was (30⁰C) same for the samples I to IV while it was 32⁰C for the fifth soil sample. Among the five sediment samples total eight strains were isolated using the Horikoshi Agar. Isolates were labeled as BC1, BC2, BC3, BC4, BC6, BC7 and BC8. All the isolates were identified by using Bergey's Manual of Determinative Bacteriology (Ninth Edition).The results are mentioned in table 1.

On the basis of cultural, morphological and biochemical characteristics as per Bergey's Manual of Determinative Bacteriology (9th Edition), it was concluded that the isolates BC1, BC2, BC3, BC4, BC6, BC7 and BC8 belong to *Pseudomonas* species, *Staphylococcus* species, and *Streptomyces* species, *Bacillus circulans*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus alvei*, respectively.

Table 1: Identification of Bacteria isolated from soil by using Standard Morphological, Biochemical and Sugar Fermentation test

SN	Isolates code	Color	Shape	Elevation	opacity	Margin	Gm Staining	Catalase	Oxidase	Nitrate	Urease	Glucose	Starch	Gelatin	Lactose	Mannitol	Fructose	Sucrose	Indole	MR	VP	Citrate	Probable Identified sp.
								+	-	+	-	+	-	+	-	+	-	+	-	+	-		
1	BC1	Greenish	Circular	Flat	Opaque	Entire	Negative rod	+	-	-	+	A	-	+	A	-	A	+	-	+	-	-	<i>Pseudomonas</i>
2	BC2	Orange	Circular	Flat	Opaque	Entire	Positive cocci	+	+	+	+	A	-	-	A	A	A	A	-	+	-	-	<i>Staphylococcus</i>
3	BC3	Off-White	Filamentous	Flat	Opaque	Entire	Positive rods	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	<i>Streptomyces</i>
4	BC4	Whitish	Circular	Flat	Opaque	Irregular	Positive rod	+	+	+	+	A	+	+	-	-	-	-	-	-	+	+	<i>Bacillus circulans</i>
5	BC5	Whitish	Circular	Flat	Opaque	Irregular	Positive rod	+	+	+	+	A	-	-	-	-	-	-	-	-	+	+	<i>Bacillus subtilis</i>
6	BC6	Whitish	Circular	Flat	Opaque	Irregular	Positive rod	+	+	+	+	A	+	+	-	-	-	-	-	-	+	+	<i>Bacillus licheniformis</i>
7	BC7	Whitish	Circular	Flat	Opaque	Irregular	Positive rod	+	+	+	+	A	+	+	-	-	-	-	-	-	+	+	<i>Bacillus megaterium</i>
8	BC8	Whitish	Circular	Flat	Opaque	Irregular	Positive rod	+	+	+	+	A	+	+	-	-	-	-	-	-	+	+	<i>Bacillus alvei</i>

Distance Decay Analysis of the Alkaliphilic isolates

The isolates were grown on the Horikoshi Agar (pH11-7) from the respective soil samples and the bacterial count (CFU/mL) was estimated. Simpson's Index of Diversity (1-D) was calculated of each isolate in all five soil samples (Table 2).

Table 2: Simpson's Index of Diversity (1-D) of bacterial isolates from different samples

Sr. No.	Sample No.	pH	BC1	BC2	BC3	BC4	BC5	BC6	BC7	BC8
1	I	11	0.9879	0.995	0.99	0.947	0.987	0.99	0.967	0.983
2	II	10	0.600	0.983	0.991	0.971	0.985	0.99	0.989	0.9921
3	III	9	0.471	0.980	0.990	0.822	0.985	0.99	0.993	0.9951
4	IV	8	0.324	0.967	0.985	0.99	0.977	0.89	0.999	0.999
5	V	7	0.271	0.9647	0.711	0.9938	0.850	0.98	0.999	0.999

Note: The less the value of Simpson's Index of Diversity (1-D) more is the diversity.

The isolates BC1, BC2, BC3, BC5 and BC6 showed increase in the diversity as we goes away from the bank (Soil sample I) of the lake towards the periphery of the lake (Soil sample V) (Fig 1). On contrary to this the diversity of the isolates BC4, BC7 and BC8 were decreasing (Fig. 2).

DISCUSSION

The ecology and diversity of various soda lakes had been studied for its biotechnological potential, ecological applications etc. The migration pattern of the species BC1, BC2, BC3, BC5 and BC6 based on the values of diversity indices showed that these species have less adaptability against high alkaline conditions as the diversity was increasing with decreasing pH. This indicates that these species are migrating away from the proximity of the Lake towards the environment where pH is less alkaline. The count of the isolate BC4, BC7 and BC8 was decreased and it indicates that these species are more adapted to the extreme pH conditions and inhabited near the bank of Lake where the conditions are more alkaline (Fig. 2). Basic results of the study agree with the Distance Decay studies carried out extensively in case of the eukaryotic organisms as well as other form of the prokaryotes such as diatoms (Carlos 2012).

CONCLUSION

The study demonstrates that the isolates from the Lonar Lake followed Distance Decay hypothesis. The results also showed that microorganism's like macro-organisms exhibit different biogeographic patterns. The growth pattern and dispersal ability are the important factors for the Distance Decay relationship. Isolates BC1, BC2, BC3, BC5 and BC6 migrating away from the lake while the isolates BC4, BC7 and BC8 made adaptations against the extreme alkaline conditions and restricted their habitat near to the lake in more alkaline conditions.

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Fig. 1: Simpson's Index of Diversity (1-D) of the isolates BC1, BC2, BC3, BC5 and BC6.

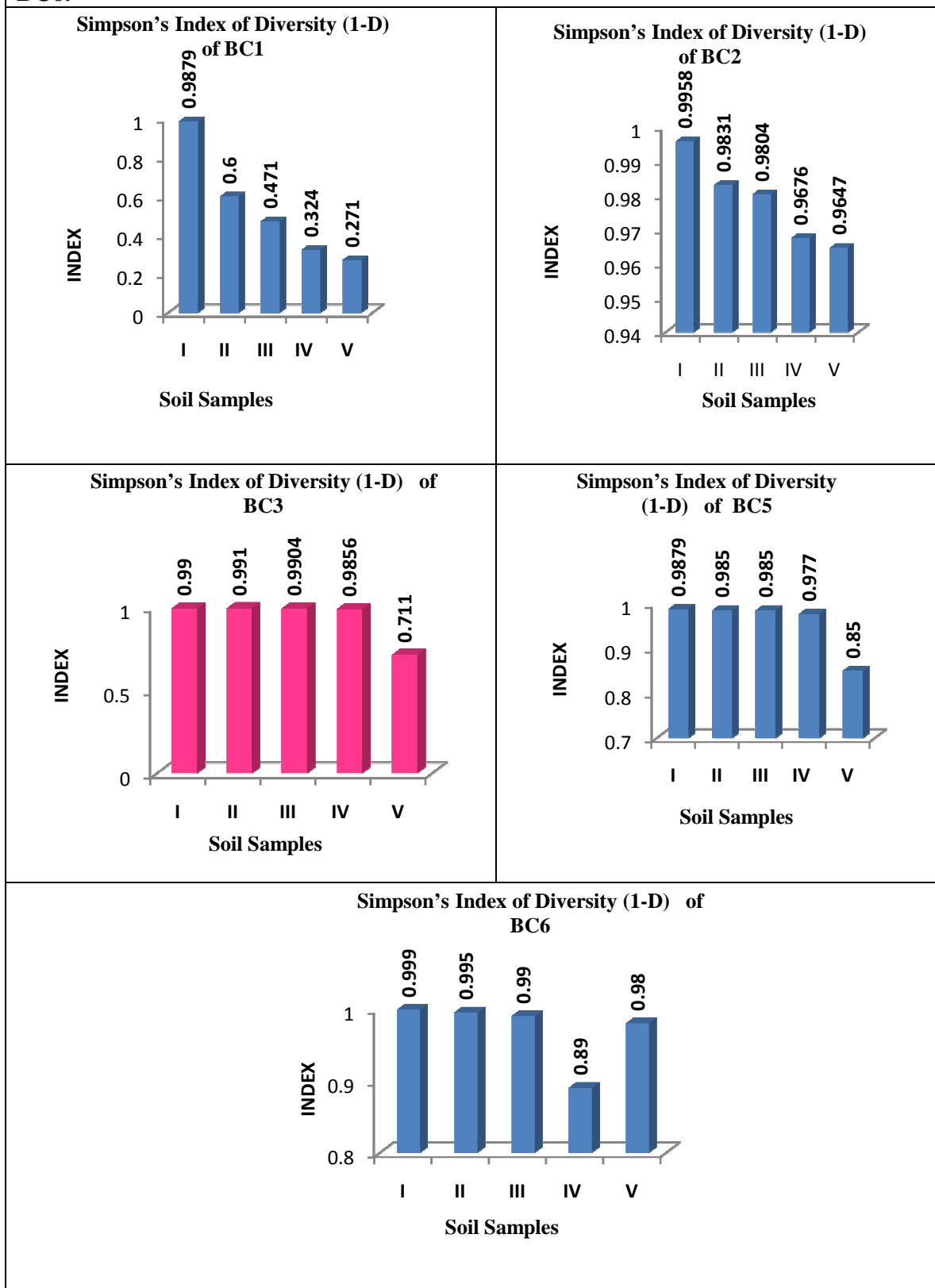
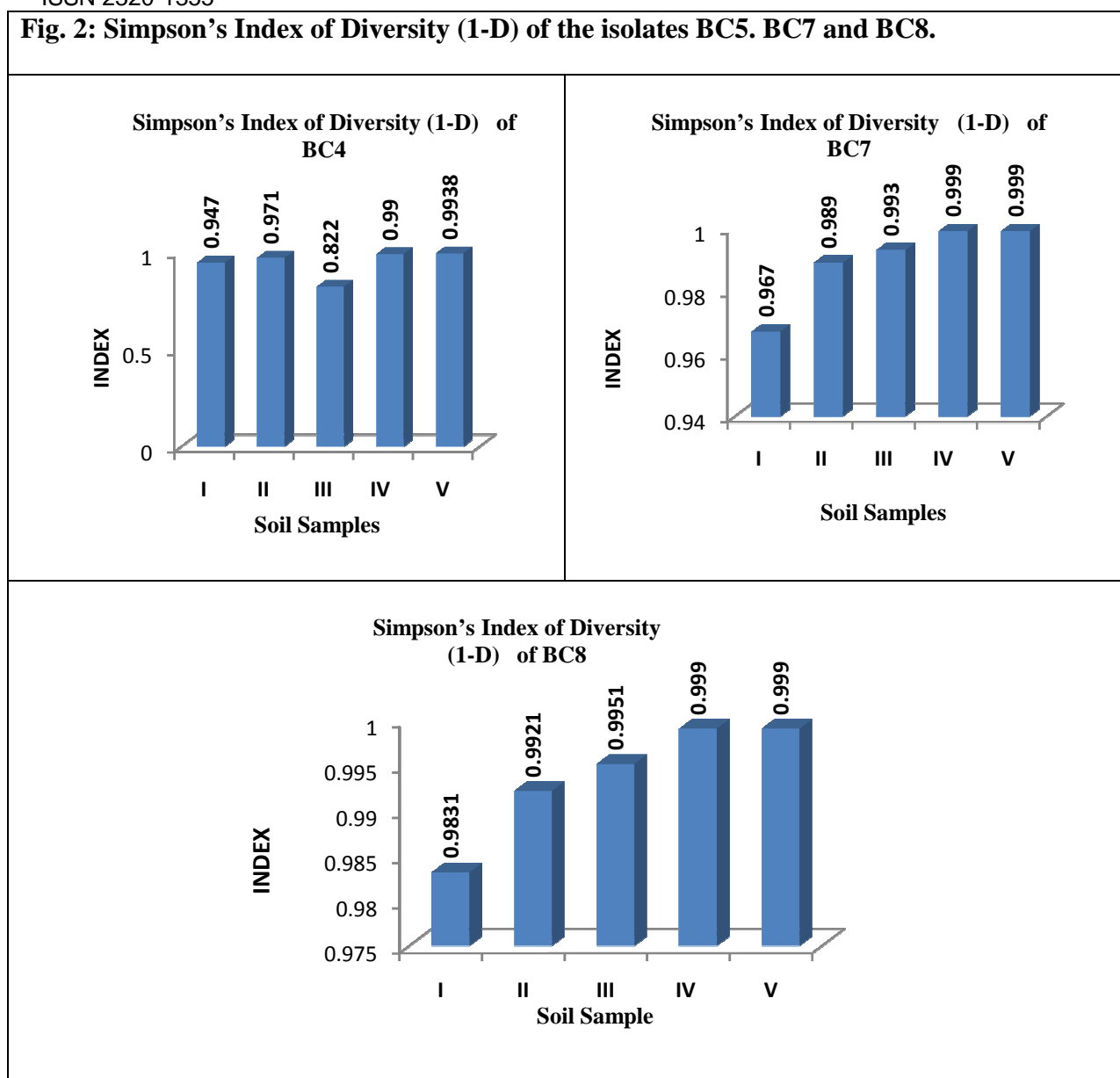


Fig. 2: Simpson's Index of Diversity (1-D) of the isolates BC5, BC7 and BC8.



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